MULTIVARIATE ANALYSIS OF GENETIC DIVERGENCE IN WHEAT (TRITICUM AESTIVUM) GERMPLASM

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Abstract

Sustainable production of food crops relies on germplasm improvement and genetic diversity. The use of multivariate techniques is an important strategy for germplasm classification and study of genetic relationships among genotypes. Wheat germplasm comprising of 50 genotypes contributed by CIMMYT was evaluated for seven quantitative traits through cluster and principal component analyses. The first three PCs with eigen values >1 contributed 70.59% of the variability amongst genotypes. The characters contributing more positively with PC1 were number of spikelets per spike, spike length and grain yield (gram per plot). The cluster analysis sequestrated 50 genotypes into 5 clusters based on Ward's method. The cluster IV and V were more clearly separated than cluster I, II and III. The cluster analysis revealed that considerable variation existed among genotypes that could be implicated in selection of wheat for the development or improvement of cultivars and germplasm.

Introduction

Wheat (*Triticum aestivum* L.) has been a staple food of the major civilizations since 8000 years. It is the most widely known grown cereal crop in the world, occupying 17% of crop acreage world over, feeding about 40% of world population and providing 20% of the total food calories and protein in human nutrition (Gupta *et al.*, 2005). Variety of foods that include bread, chapattis, cakes, cookies and confectionary items are made from it. In Pakistan, it is cultivated on largest acreage. It was cultivated on an area of 9.062 million hectare during 2008-09 with a production of 23.421 million tons, contributing 31% to the value added in agriculture and 2.8% to the GDP (Anon., 2009).

Germplasm improvement and genetic diversity is a key to reliable and sustainable production of the food crops. For effective evaluation and utilization of germplasm, measure of extent of available genetic diversity is of utmost importance (Zubair *et al.*, 2007). The use of multivariate statistical algorithms is an important strategy for classification of germplasm and analysis of genetic relationships among breeding material (Mohammadi & Prasanna, 2003).

Fufa et al., (2005) clustered hard red winter wheats on the basis of pedigree information, morphological characters, end-use quality traits and molecular markers to estimate genetic diversity. Leilah & Al-Khateeb (2005) used seven statistical procedures to study the relationship between wheat grain yield and its components. Ahmad et al., (2008) evaluated 113 accessions of barley by using cluster and principal component analysis. Seventy wheat genotypes were evaluated for variability parameter including cluster analysis for eight traits by Ali et al., (2008). Aharizad et al., (2012) applied cluster analysis using Wards algorithm and squared Euclidean distances and assigned 94 bread wheat inbred lines into three groups. The level of variation was higher for peduncle length, flag leaf area, number of spikes and grain yield. Soleymanfard et al., (2012) reported that 75% variation in grain yield was due to spikes m², 1000 grain weight and plant height. Present study was conducted to decipher the extent of genetic variation and relationships among wheat genotypes based on quantitative traits using multivariate analysis and to identify the set of morpho-agronomic attributes which could be further utilized in breeding programs.

Material and Methods

Plant material for the study comprised of Elite Spring Wheat Yield Trial (ESWYT), consisted of 50 advance lines of bread wheat (rainfed) contributed by CIMMYT (Table 1). ESWYT is targeted to continental low rainfall areas (average 250-400 mm) with relatively low winter temperatures. The experiment was planted at National Agricultural Research Centre, Islamabad, Pakistan during 2007-08 following alpha lattice design with two replications split with 10 blocks having 5 plots per block containing five different genotypes. Each plot consists of 6 rows, 5m long and 25 cm apart. Fertilizer was applied at the rate of 90:90kg NP respectively with no irrigation. The data recorded were days to heading, days to maturity, plant height (cm), spike length (cm), number of spikelets per spike, 1000 grain weight, yield (gram per plot).

The combined data of grain yield and its components were then subjected to analysis to estimate the simple statistic i.e., mean, range, variance, standard error and simple correlation. Quantitative traits were analyzed by cluster and principal component analysis with the help of software program 'Statistca' v 6.0 and 'SPSS' v 12.0 for windows. Cluster analysis identifies variable which are further clustered into main group and subgroups using Ward's method. The genotypes in each cluster were also analyzed for basic statistics. Principal component analysis simplifies the complex data by transforming number of correlated variables into a smaller number of variables called principal components. The first principal component accounts for maximum variability in the data as compared to each succeeding component. Scatter diagram was plotted to show the variation pattern. Mean value of each variable were standardized prior to cluster and principal component analysis to avoid the effects due to difference in scale.

Entry No	Construes
<u>1</u>	GA 2002 (check)
1	DDW/242
2	FDW 343 SEDI / DAVON
3	SERI/RATON HD2687
4	
5	PBW450 MUDCA
0	
/	BL1490 / MILAN / 5 / CROC_1 / AE.SQUARKOSA (205)//
8 0	HPO/ IAN// VEE/ 5/ 2 * POO/ 4/ MILAN/ 5/ SSEKII MILAN/ 887220 // DADAX
9	MILAN / 50/230 // DADAA MILAN / 579220 // DADAX
10	MILAN / 578230 // BABAA MILAN / 578230 // BABAA
11	MILAN / 5/8230 // BABAA
12	KAUZ // ALTAR 84 / AOS / 5 / MILAN / KAUZ / 4 / HUITES
13	KAUZ // ALTAK 84 / AUS / 5 / MILAN / KAUZ / 4 / HUITES
14	$WEAVEK / 5 / SAP1 / IEAL // HUI / 4 / CKUC _ 1 /$
15	CS / IH.SC // 5 * PVN / 5 / MIRLO / BUC / K / MILAN / 5 / IILHI
10	AUZ = 2 / MINV / AUZ = 3 / MILAN / 4 / BABAA
17	ATTELA / $3 + DCN / / DAV 92 / 3 / TASTOK$
10	ATTILA / $5 + DCIN + 2 // DAV 92$
19	KEIUFA / FASIOK // BADAA CAD // VAL / DD / 2 / NAC / 4 / VEE / DCN // 2* THL / 5 / MILAN
20	CAR // RAL / BD / 5 / NAC / 4 / VEE / POIN // 2* 101 / 5 / MILANTOP A 07 / DASTOD
21	TODA 97 / PASTOR
22	$\frac{100A 977 FASTOK}{CAL} = \frac{100}{100} $
23	CAL / NH // HS 07.717 S7 SER174 / CAL / NH // HS07.717 S7
24	OASIS / SKAUZ // A * BCN / 3 / PASTOR / A / KAUZ * 2 / VACO //
25	BABAY / 3 /OASIS / SKAUZ // 4 BCN / 4 / RACE 2/ TACO //
20	IRENA / 2 * PASTOR
28	PRINIA / PASTOR
29	INOALAB 91 * 2 / KUKANA
30	INQALAB 91 * 2 / TUKURU
31	BABAX // IRENA / KAUZ / 3 / HUITES
32	OASIS / SKAUZ // 4 * BCN / 3 / 2 * PASTOR
33	DVERD 2 AE SOUARROSA (221) / 3 / URES / BOW //
34	FRET2 / WBLL1 // KAMB1
35	KAMB1 / MNNK1 // WBLL1
36	FRET2 * 2 / 4 / SNI / TRAP#1 /3 / KAUZ * 2 / TRAP // KAUZ
37	FRET2 / TUKURU // FRET2
38	FRET2 / TUKURU // FRET2
39	FRET2 / TUKURU // FRET2
40	WBLL1 / 3 / STAR // KAUZ / STAR / 4 / BAV 92 / RAYON
41	ALTAR 84 / AE.SQUARROSA (221) // 3 * BORL 95 /3 /
42	TUKURU // BAV 92 / RAYON
43	TUKURU // BAV 92 / RAYON
44	SUNSU / CHIBIA
45	KAUZ / PASTOR // PBW343
46	WBLL1 * 2 / KKTS
47	WBLL1 * 2 /4 YACO / PBW65 / 3 / KAUZ * 2 / TRAP // KAUZ
48	WBLL1 * 2 /4 YACO / PBW65 / 3 / KAUZ * 2 / TRAP // KAUZ
49	WBLL1 * 2 / VIVITSI
50	WBLL1 * 2 / VIVITSI

Table 1. Genotypes in Elite Spring Wheat Yield Trial.

Results and Discussion

Basic statistics for quantitative traits are presented in Table 2 that showed a considerable variability in the material under study. Medium to high variance was observed for 1000 grain weight, days to heading and days to maturity, plant height and yield (gram per plot). A small genetic variance was observed for number of spikelets per spike and spike length. Improvement of these traits through simple selection might be limited from germplasm used in the present study.

Simple correlation analysis: A matrix of simple correlation coefficients between grain yield and its components were computed and presented in Table 3. Results revealed that 1000 grain weight have positive association with days to heading and days to maturity, plant height, spike length and significant positive association with number of spikelets per spike and yield (gram per plot) suggesting the need of more emphasis on these components for increasing the grain yield in wheat. Leilah & Khateeb (2005) showed significant positive association between 100 grain weight and grain yield and Ali et al., (2008) showed positive genotypic and phenotypic correlation of 1000 grain weight with yield per plant. Days to heading showed a significant positive correlation with days to maturity and negative association with spike length and yield (gram per plot). Days to maturity showed negative association with plant height, spike length and yield (gram per plot) except number of spikelets per spike. Plant height showed positive association with number of spikelets per spike and spike length, and no correlation with yield (gram per plot). Leilah and khateeb (2005) showed a significant positive relationship of plant height with grain yield. However, Ali et al., (2008) showed a significant negative genotypic and phenotypic correlation of plant height with yield per plant. Number of spikelets per spike showed a significant positive relation with spike length. Positive association with yield (gram per plant) was also reported by Ali et al., (2008), whereas spike length showed negative association with yield (gram per plot).

Cluster and principal component analysis: In order to maintain, evaluate and utilize germplasm effectively, it is important to investigate the extent of genetic diversity available. Morphological traits have been successfully used for estimation of genetic diversity and cultivar development since they provide a simple way of quantifying genetic variation (Fufa *et al.*, 2005). Analysis of genetic diversity in germplasm collection aids in classification of genotypes and identification of core collections with possible utility for specific breeding goal (Mohammadi & Prasanna, 2003). The cluster analysis sequestrates genotypes into clusters which exhibit high homogeneity within a cluster and high heterogeneity between clusters (Jaynes *et al.*, 2003).

Cluster analysis sequestrated 50 genotypes of wheat into 5 clusters (Fig. 1). Members of each cluster are presented in Table 4. Cluster I consisted of 6 genotypes, cluster II of 17 genotypes, cluster III of 4 genotypes, cluster IV of 13 genotypes and cluster V of 10 genotypes. Though cluster analysis grouped genotypes together with greater morphological similarity, the clusters did not necessarily include all genotypes from same origin. Zubair et al., (2007), Ahmad et al., (2008) and Ali et al., (2008) also reported lack of association between morphoagronomic traits and origin. Mean values along with standard deviation for each cluster (Table 5) revealed that genotypes in the cluster I showed earlier maturity where as genotypes in cluster V were late maturing and hence the representative genotypes of cluster I can be used for induction of earliness. The genotypes in the cluster II can be used for the improvement of plant height. The genotypes in cluster I, II, IV, V showed low to medium yield potential. The genotypes in cluster I showed lowest vield but maximum spike length. The genotypes in cluster III (V4, V5 V12 and V23) showed maximum 1000 grain weight, yield (gram per plot) and number of spikelets per spike, and therefore these genotypes could be exploited for their direct release as a variety (s) after testing under wide range of environments. Moreover, these genotypes can also be used as parents in hybridization programs to develop high yielding wheat varieties.

Tuble 2. Duble studbles for seven quantitative traits of 50 wheat genotypes.								
S. No.	Traits	Mean ± S.D	Maximum value	Minimum value	Variance			
1.	1000 grain weight (g)	21.120 ± 3.6681	15.000	34.000	13.45			
2.	Days to heading	165.380 ± 2.2668	162.000	169.000	5.14			
3.	Days to maturity	119.980 ± 2.2451	114.000	125.000	5.04			
4.	Plant height (cm)	87.780 ± 5.9635	74.000	100.000	35.56			
5.	No. of spikelets per spike	19.855 ± 1.9611	15.000	25.000	3.85			
6.	Spike length (cm)	10.312 ± 0.9630	8.500	12.470	0.93			
7.	Yield (gram per plot)	1516.400 ± 190.7243	1038.000	2045.000	36375.76			

Table 2. Basic statistics for seven quantitative traits of 50 wheat genotypes.

Table 3. A matrix of o	correlation (coefficients	(r) for the e	estimated s	even variabl	es of wheat.	
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Variables	X1	X2	X3	X4	X5	X6	X7
1000 garin weight (g) (X1)		0.13	0.02	0.03	0.40**	0.14	0.47**
Days to heading (X2)			0.56**	0.05	0.07	-0.04	-0.17
Days to maturity (X3)				-0.08	0.03	-0.20	-0.05
Plant height (cm) (X4)					0.03	0.11	0.00
No. of spikelets per spike (X5)						0.72**	0.21
Spike length (cm) (X6)							-0.07
Yield (gram per plot) (X7)							

** Significant at 1% level of probability

Cluster	Frequency	Cluster memberships
Ι	6	V15, V29, V37, V38, V39, V50
II	17	V9, V11, V13, V18, V21, V22, V25, V30, V34, V36, V41, V42, V43, V44 V46, V47, V48
III	4	V4, V5, V12, V23
IV	13	V2, V3, V6, V7, V8, V10, V14, V16, V17, V19, V24, V28, V49
V	10	V1, V20, V26, V27, V31, V32, V33, V35, V40, V45

Table 4. Five cluster grouping wheat genotypes based on seven quantitative traits.

Table 5. Mean and standard deviation for five clusters based on seven quantitative traits.

Traits	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V
1000 grain weight (g)	18.84 ± 3.19	22.25 ± 2.96	27 ± 5.29	18.31 ± 2.35	21.9 ± 1.37
Days to heading	162.67 ± 1.21	165.76 ± 1.56	164 ± 1.83	164.46 ± 1.89	168.1 ± 1.19
Days to maturity	117 ± 1.89	119.71 ± 1.86	119.75 ± 0.95	119.76 ± 1.31	122.6 ± 1.71
Plant height (cm)	85.17 ± 6.65	91.71 ± 4.14	86.75 ± 6.89	88 ± 5.71	82.8 ± 4.19
No. of spikelets per spike	20.27 ± 2.15	20.54 ± 1.41	21.95 ± 0.89	17.78 ± 1.25	20.31 ± 1.75
Spike length (cm)	11.46 ± 0.81	10.69 ± 0.73	10.62 ± 0.54	9.32 ± 0.67	10.14 ± 0.61
Yield (gram per plot)	1369.83 ± 124.8	1501.41 ± 106.7	1922.5 ± 124.2	1546.31 ± 208.9	1428.5 ± 28.04

Table 6. Princip	al Component	t analysis of	wheat genotypes
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		PC ₁	PC ₂	PC ₃
Eigen value		2.007	1.612	1.322
Proportion of variance		28.671	23.031	18.884
Cumulative variance		28.671	51.702	70.586
	Communalities		Eigenvectors	
1000 grain weight	0.737	0.660	0.231	0.498
Days to heading	0.798	-0.039	0.881	-0.144
Days to maturity	0.771	-0.162	0.862	0.048
Plant height	0.069	0.148	-0.038	-0.213
No. of spikelets per spike	0.855	0.879	0.154	-0.242
Spike length	0.899	0.739	-0.100	-0.586
Yield (gram per plot)	0.812	0.451	-0.077	0.777

Principal component analysis simplifies the complex data by transforming the number of correlated variables into a smaller number of variables called principal components. The first principal component accounts for maximum variability in the data with respect to succeeding components (Leilah & Al-Khateeb, 2005). The analysis had grouped the estimated wheat variables into three main components. The first three components with eigen values >1 accounted for 70.59% of the total variation of grain yield. PC1 accounted for about 28.67% of the variation in grain yield; PC2 for 23.03% and PC3 for 18.88% (Table 6). The first PC was related to yield and yield contributing traits whereas the second and third PC contrasts variables that related solely to vegetative growth. The traits, which contributed more positively to PC1 were number of spikelets per spike, spike length and yield (gram per plot), suggesting that this component reflected the yield potential of each genotype. The first two principal components contributing more than half of the variance were plotted to observe relationships

between the clusters (Fig. 2). Cluster IV and V showed considerable clear separation than cluster I, II and III which might be due to mixture of genotypes with different taxonomic traits grouped in these cluster.

Increased yield potential is a stated goal for plant breeders. Progress in yield potential results from the progressive accumulation of genes conferring higher yield or elimination of the unfavorable genes through the breeding process. The present study revealed that 1000 grain weight had strong correlation with number of spikelets per spike and yield, suggesting the need of more emphasis on these components for increasing the grain yield in wheat. Grouping of genotypes by multivariate methods in the study is of practical value for the wheat breeders. Representative genotypes may be chosen from the particular groups for hybridization programs with other approved cultivars. This will aid in identification, selection and combining genotypes to obtain important traits in one line with a broad genetic base.



Fig. 2. Scatter diagram of wheat genotypes for first two PCs.

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